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Research Focus

Matrix metalloproteinase-dependent EGF receptor activation in hypertension and left ventricular hypertrophy

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Agonist stimulation of certain G protein-coupled receptors (GPCRs) causes shedding of heparin-binding epidermal growth factor (HB-EGF) through activation of matrix metalloproteinases (MMPs), with subsequent transactivation of the EGF receptor. MMPs are widely expressed, and their dysregulated expression is crucial in cancer, inflammation, and cardiovascular remodeling. Recent studies in hypertensive animals have shown enhanced expression and activation of MMPs and EGF receptors, and their inhibition attenuates cardiac hypertrophy, vasoconstriction and hypertension induced by GPCR agonists such as angiotensin II, endothelin-1 and phenylepherine. These findings suggest that selective inhibition of MMPs might have therapeutic potential in hypertension and other cardiovascular diseases.

The extracellular matrix (ECM) is recognized as a crucial regulatory component in providing an environment for cell signaling, cell–cell interactions and cell migration, division, differentiation, anchorage and survival or death. The basic structural elements of the ECM include: collagen, elastin, and more specialized proteins such as fibrillin, fibronectin, and proteoglycans. The highly regulated control of ECM homeostasis is governed, in part, by the action of a specific class of proteolytic enzymes known as matrix metalloproteinases (MMPs), a group of zinc- and calcium-dependent enzymes. MMPs are synthesized as zymogens with a signal sequence and a propeptide segment that is removed during activation. Once activated, certain MMPs are secreted into the ECM whereas others, the membrane type (MT-MMPs), remain tethered to the cell surface by a hydrophobic transmembrane domain. The main function of MMPs seems to be tissue remodeling, and they are involved in a variety of processes, including wound healing, embryonic development, nerve growth, angiogenesis, and the release of defense proteins and growth factors. Under normal conditions their proteolytic activity is precisely regulated by their endogenous protein inhibitors, termed tissue-specific inhibitors of MMPs (TIMPs). Disruption of the balance between MMPs and TIMPs contributes to pathophysiological processes such as arthritis, tumor growth, and metastasis. The MMPs and their inhibitors also have a crucial role in the regulation of the composition of the ECM during cardiac and vascular remodeling, and hypertension. MMPs have also been implicated in the genesis of myocardial infarction, left ventricular dilatation, and heart failure. Over 26 MMPs have been identified and classified based on their specificity for substrates such as collagenase and gelatinase [1–3].

Regulation of cardiovascular function by metalloproteinases

Much attention has focused on the molecular mechanisms that mediate the adverse effects of G protein-coupled receptor (GPCR) stimulation in the cardiovascular system (CVS). A major recent development in this area was the finding that many of the proliferative and mitogenic effects of GPCRs are mediated through transactivation of the epidermal growth factor receptor (EGF-R) [4,5]. Many ligands, including heparin-binding EGF (HB-EGF), for the EGF receptor family are shed from cell surfaces by MMP activation in response to specific signals, leading to phosphorylation of the EGF-R (EGF-R transactivation) and initiation of downstream mitogen activated protein kinase (MAPK) activation [4].

Arterial hypertension is one of the major cardiovascular risk factors in stroke and heart disease. Increased hemodynamic stress, from both pressure and volume overload, is fundamental to the development of left ventricular hypertrophy (LVH), which is characterized by alterations in the structure and muscle content of the heart and blood vessels [3]. The hemodynamic changes in the CVS are mediated by a variety of extracellular stimuli, such as mechanical stress, GPCR agonists, cytokines and growth factors. Sustained stimulation of GPCRs such as those for adrenergic ligands, angiotensin II (Ang II) and endothelin-1 (ET-1) can culminate in hypertension, vascular inflammation and atherosclerosis, tissue remodeling, and LVH [6,7].

Whereas substantial evidence supports a general role of MMPs in the control of many aspects of cardiovascular function that require tissue remodeling and cell growth, their direct involvement in GPCR-mediated hypertension has been shown only recently. Hao *et al.* [8] have studied the role of EGF-R activation through MMP-dependent shedding of HB-EGF *in vitro* as well as in hypertensive rats. Stimulation of α_{1b}-adrenoceptors with phenylepherine causes EGFR activation through MMP7 in rat mesenteric arteries. Inhibition of EGFR with AG1478, of HB-EGF shedding with CRM197, or of MMP activation

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with GM6001 and doxycycline, prevents adrenergic vasoconstriction. In spontaneously hypertensive rats (SHR), MMP levels are higher at all time points compared with controls. Administration of doxycycline, the only clinically approved MMP inhibitor [9], reduces systolic blood pressure and attenuates HB-EGF shedding in mesenteric arteries of SHR but not in normotensive rats. Interestingly, protein kinase (PKB, Akt) and p38, but not ERK1/2, contribute to arterial adrenergic tone, possibly through an interaction with cytoskeletal or scaffolding proteins. These data suggest a role for MMP-7-mediated HB-EGF shedding in the development and progression of hypertension. Similar results were obtained with Ang II treatment (Figure 1). Previous reports have shown high levels of EGF-R and phosphorylated ERK1/2 in young SHR. Administration of antisense oligodeoxynucleotide to EGF-R decreases LV weight or body weight and blood pressure in young but not in adult SHR, suggesting a crucial role of the EGFR-activated ERK1/2 pathway in cardiovascular development but not in the maintenance of established LVH in adult animals [10].

MMPs are responsible for agonist-induced EGF receptor transactivation

EGF has been found to be a potent vasoconstrictor in the thoracic aorta of hypertensive rats [11]. The EGF-R, a receptor tyrosine kinase, is endogenously expressed in numerous cell types and is an important factor in the control of many fundamental cellular processes, including

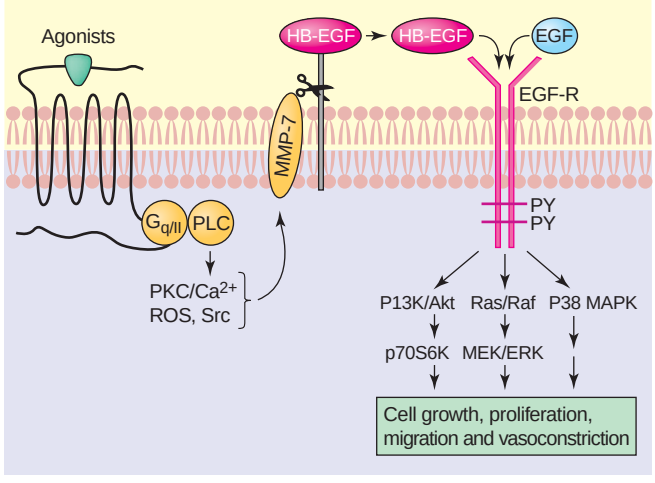

Figure 1. Signaling pathways activated by G protein-coupled receptor (GPCR) agonists such as Ang II, ET-1 and phenylepherine. Binding of agonists to their heptahelical receptors causes activation of phospholipase C-β1 (PLC) and generation of diacylglycerol and inositol triphosphate, which in turn cause activation of PKC and release of Ca^{+2} from intracellular stores. Intracellular signaling events such as generation of reactive oxygen species (ROS) and phosphorylation of Src cause activation of MMP-2 and -9 in the heart and MMP-7 in arteries, which leads to processing of pro-heparin binding EGF to HB-EGF. The latter activates the EGF receptor, leading to phosphorylation of phosphoinositide 3-kinase (PI3K), Akt and MAP kinases including ERK1/2 and p38 MAP kinase. ERK1/2 and Akt are required for the development of LVH, whereas p38 MAPK and Akt contribute to adrenergic tone in rat mesenteric arteries. Abbreviations: EGF, epidermal growth factor; EGF-R, epidermal growth factor receptor; HB-EGF, heparin-binding epidermal growth factor; MAPK, mitogen activated protein kinasse; MMP, matrix metalloproteinase; PKS protein kinase C; PY, tyrosine phosphorylation.

the cell cycle, cell migration, cell metabolism and survival, and cell proliferation and differentiation [12]. Overexpression and/or overstimulation of the EGF-R by MMP-induced HB-EGF can lead to dysregulated growth and tissue remodeling. Several recent studies have demonstrated that blockade of MMPs and a disintegrin and metalloproteinase (ADAMs) with pharmacological inhibitors, or with their dominant negative mutants, abolishes EGF-R transactivation, MAPK phosphorylation and hypertrophic responses in response to stimulation with various GPCR agonists [13–15].

Studies using native vascular smooth muscle cells (VSMCs) and targeted overexpression of the type 1 Ang II receptor (AT_1-R) in cardiomyocytes suggest that Ang II can directly promote growth of these cells via transactivation of the EGF-R and subsequent activation of MAPKs. This process is mediated by the production of HB-EGF by MMPs. Blockade of the generation of HB-EGF by MMP inhibitors, or abrogation of EGF-R kinase by selective pharmacological inhibitors or antisense oligonucleotides, protects against Ang II-mediated cardiac hypertrophy [14–16] and VSMC migration [17]. Similarly, the MMP inhibitor, KB-R7785, prevents Ang II-induced EGF-R transactivation and cardiac hypertrophy. Moreover, dominant-negative expression of ADAM12 abrogates Ang II-induced cardiac hypertrophy through inhibition of HB-EGF shedding [15], suggesting that blockade of MMPs by specific inhibitors could prove to be a potent therapeutic strategy.

Endothelin-1 (ET-1), a pleiotropic hormone produced primarily by the endothelium, is a potent vasoconstrictor and its synthesis is stimulated by vasoactive agents (Ang II and norepinephrine), cytokines (e.g. tumor necrosis factor-α) and mechanical stress. Using aortas isolated from transgenic mice harboring the luciferase gene under control of the collagen 1-α 2 chain promoter, Flamant *et al.* [18] showed that ET-1-induced activation of collagen-1 gene expression and its acute pressor effect are mediated through activation of the EGF-R. Administration of ET-1 (i.v.) causes a strong and sustained increase in blood pressure that is inhibited by the EGF-R antagonist, PD153035. Pharmacological blockade of the EGF-R also blunts the vasoconstrictor action of ET-1 in isolated aortic rings and anesthetized animals, an effect similar to that observed in transgenic mice with decreased EGF-R function, indicating that fibrogenic and contractile actions of ET-1 are mediated through the EGF-R.

Ang II is the dominant effector of the rennin–angiotensin system (RAS) and has important physiological actions on blood pressure regulation and aldosterone secretion. However, over-stimulation of the RAS can result in hypertension, atherosclerosis, tissue remodeling, and LVH. The pathogenesis of LVH is linked to activation of the RAS, since angiotensin converting enzyme (ACE) inhibitors and AT_1-R antagonists such as losartan, valsartan and candesartan can reverse LVH in hypertensive patients [19]. More recently, Sakata *et al.* [20] performed studies to determine whether activation of MMPs precedes, or is secondary to, LV remodeling. For this purpose, a hypertensive heart failure (HF) model of Dahl salt-sensitive rats fed a high-salt diet for 8 weeks was

used. Such HF animals show elevated expression of MMP-2 and MMP-9 at 23 weeks without LV dilatation. However, LV dilatation, LV systolic dysfunction, and pulmonary edema occur at 26 weeks with further enhancement of the expression and activity of MMPs. Administration of the ACE inhibitor (enalarpil, 5 mg/kg/d) from 9 weeks prevents such geometrical and functional deterioration. Thus, MMPs appear to promote LV remodeling, and ACE blockade inhibits MMP activation by reducing Ang II formation, thereby preventing LV remodeling and dysfunction. These recent *in vitro* and animal studies have provided promising findings on the reduction of hypertension and LVH through blockade of MMPs and the EGF-R. The extent to which inhibition of these molecules is clinically applicable to the prevention and treatment of hypertension and LVH in human patients remains to be determined.

References

1 Armiento, J.D. (2002) Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. *Trends Cardiovasc. Med.* 12, 97–101
2 Spinale, F.G. (2002) Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ. Res.* 90, 520–530
3 Tayebee, M.H. *et al*. (2003) Extracellular matrix biology: a new frontier in linking the pathology and therapy of hypertension? *J. Hypertens.* 21, 2211–2218
4 Prenzel, N. *et al*. (1999) EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* 402, 884–888
5 Shah, B.H. and Catt, K.J. (2003) A central role of EGF receptor transactivation in angiotensin II-induced cardiac hypertrophy. *Trends Pharmacol. Sci.* 24, 239–244
6 Rockman, H.A. *et al*. (2002) Seven-transmembrane-spanning receptors and heart function. *Nature* 415, 206–212
7 Virdis, A. and Schiffrin, E.L. (2003) Vascular inflammation: a role in vascular disease in hypertension? *Curr. Opin. Nephrol. Hypertens.* 12, 181–188
8 Hao, L. *et al*. (2004) Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. *Circ. Res.* 94, 68–76
9 Peterson, J.T. (2004) Matrix metalloproteinase inhibitor development and the remodeling of drug discovery. *Heart Fail. Rev.* 9, 63–79
10 Kagiyama, S. *et al*. (2003) Antisense to epidermal growth factor receptor prevents the development of left ventricular hypertrophy. *Hypertension* 41, 824–829
11 Florian, J.A. and Watts, S.W. (1999) Epidermal growth factor: a potent vasoconstrictor in experimental hypertension. *Am. J. Physiol.* 276, H976–H983
12 Schlessinger, J. (2002) Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* 110, 669–672
13 Seals, D.F. and Courtneidge, S.A. (2003) The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* 17, 7–30
14 Thomas, W.G. *et al*. (2002) Adenoviral-directed expression of the type 1A angiotensin receptor promotes cardiomyocyte hypertrophy via transactivation of the epidermal growth factor receptor. *Circ. Res.* 90, 135–142
15 Asakura, M. *et al*. (2002) Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metallproteinase inhibitors as a new therapy. *Nat. Med.* 8, 35–40
16 Eguchi, S. *et al*. (2001) Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metallprotease-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK. *J. Biol. Chem.* 276, 7957–7962
17 Saito, S. *et al*. (2002) Metalloprotease inhibitor blocks angiotensin II-induced migration through inhibition of epidermal growth factor receptor transactivation. *Biochem. Biophys. Res. Commun.* 294, 1023–1029
18 Flamant, M. *et al*. (2003) Epidermal growth factor receptor transactivation mediates the tonic and fibrogenic effects of enodthelin in the aortic wall of transgenic mice. *FASEB J.* 17, 327–329
19 de Gasparo, M. *et al*. (2000) International Union of Pharmacology: XXIII, The angiotensin II receptors. *Pharmacol. Rev.* 52, 415–472
20 Sakata, Y. *et al*. (2004) Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats. Its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. *Circulation* 109, 2143–2149

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Flying through the genome: a comprehensive study of functional genomics using RNAi in *Drosophila*

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Sequencing the DNA of an entire mammalian genome now seems routine. The human sequence along with the mouse – the model for mammalian genetics – and the rat – the model for mammalian physiology – are now part of the data archive. However, the real challenges for the 21st century are what to do with this information and how to test the function of so many different genes in so many different cellular contexts. The potential payoffs are enormous. Examples include a better understanding of disease pathologies with effective strategies for therapeutic interventions that cause few, if any, side effects.

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To understand why the fruit fly, *Drosophila melanogaster*, remains an important genomic model system, it is important to remember that the formulated science of genomics did not begin at the end of the 20th century with the advent of high-throughput sequencing technologies. As outlined in Table 1, for more than 90 years the *Drosophila* community has made substantial contributions to our knowledge base of how genes are organized, expressed and regulated at the genomic level.

There are many reasons why *Drosophila* has been so popular for genomic studies (reviewed in [17]). First, the genome is organized into only four chromosomes, which was an asset for establishing the first linkage maps. A